1297. NATRONIELLA SULFIDIGENA MEDIUM

$NaHCO_3$	25.00	g
Na_2CO_3	140.00	g
NaCl	15.00	g
K_2HPO_4	1.00	g
NH_4Cl	0.20	g
$MgCl_2$ x 6 H_2O	0.20	g
Yeast extract	0.20	g
Trace element solution (see medium 1369)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Vitamin solution (see medium 141)	10.00	ml
Glycerol	2.00	g
Sulfur, powdered	2.00	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve hydrogencarbonate, carbonate, sodium chloride and hydrogenphosphate, then sparge medium with 100% N_2 gas for 30 – 45 min to make it anoxic. Dispense solution under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add ammonium chloride, magnesium chloride, yeast extract, trace elements, vitamins, glycerol and sulfide from sterile anoxic stock solution prepared under 100% N_2 gas. The vitamin solution should be sterilized by filtration. Sulfur is sterilized by steaming for 3 hours on each of 3 successive days and added aseptically to the sterile medium as powder while retaining anoxic conditions. Adjust pH of complete medium to 9.8 – 10.0, if necessary.